
Single amino acid changes that alter the DNA sequence specificity of the DNA-[N⁶-adenine] methyltransferase (Dam) of bacteriophage T4

Zoe Miner⁺, Samuel L.Schlagman and Stanley Hattman*

Department of Biology, University of Rochester, Rochester, NY 14627, USA

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ABSTRACT

Bacteriophage T4 codes for a DNA-[N⁶-adenine] methyltransferase (Dam) which recognizes primarily the sequence GATC in both cytosine-and hydroxymethylcytosine-containing DNA. Hypermethylating mutants, *dam^h*, exhibit a relaxation in sequence specificity, that is, they are readily able to methylate non-canonical sites. We have determined that the *dam^h* mutation produces a single amino acid change (Pro¹²⁶ to Ser¹²⁶) in a region of homology (III) shared by three DNA-adenine methyltransferases; viz, T4 Dam, *Escherichia coli* Dam, and the *DpnII* modification enzyme of *Streptococcus pneumoniae*. We also describe another mutant, *dam^c*, which methylates GATC in cytosine-containing DNA, but not in hydroxymethylcytosine-containing DNA. This mutation also alters a single amino acid (Phe¹²⁷ to Val¹²⁷). These results implicate homology region III as a domain involved in DNA sequence recognition. The effect of several different amino acids at residue 126 was examined by creating a polypeptide chain terminating codon at that position and comparing the methylation capability of partially purified enzymes produced in the presence of various suppressors. No enzyme activity is detected when phenylalanine, glutamic acid, or histidine is inserted at position 126. However, insertion of alanine, cysteine, or glycine at residue 126 produces enzymatic activity similar to *Dam^h*.

INTRODUCTION

The related T-even phages, T2 and T4 (but not T6), specify a DNA-[N⁶-adenine] methyltransferase (m⁶A-MTase), Dam, that methylates the A residue in the sequence, GATC(1,2,3). The normal substrate for the enzyme is 5-hydroxymethylcytosine (hmC)-containing DNA, since these viruses contain this base in place of cytosine (C); the hmC is modified further by glucosylation. Nonglucosylating mutants (*gt⁻*) are different from their *gt⁺* parents in that their DNA is restricted by certain bacterial strains, such as P1-lysogens (4,5). Derivatives of T2 *αgt⁻* and T4 *αgt⁻ βgt⁻* phage mutants capable of growth on P1-lysogens have been isolated; these are designated *dam^h* (originally *uPI*) because they exhibit hypermethylation of their DNA (6). Thus, *Dam^h*, but not *Dam⁺*, methylation protects against restriction by P1 (7) [recognition sequence, AGACC (8,9)]. This implies that the *Dam^h* enzyme has an altered DNA sequence specificity compared to *Dam⁺*.

E. coli also encodes a Dam enzyme that recognizes the sequence, GATC (8,10,11). Although comparison of the T4 *dam* and *E. coli dam* gene nucleotide sequences showed no similarity, the derived amino acid sequences revealed four regions of homology (12). A third m⁶A-MTase, which also methylates the sequence GATC, M-*DpnII* from *S. pneumoniae*, possesses three of the four homology regions (12). Since T2 *dam^h* and T4 *dam^h* mutants appear to have an altered nucleotide sequence specificity, elucidation of the

location and nature of the *dam*^h mutation should give insight into the domain(s) responsible for protein-DNA interaction. However, initial attempts to clone the *dam*^h allele proved unsuccessful. We thought that this might be due to cytotoxicity caused by high levels of methylation. Therefore, an alternative strategy was adopted that took advantage of *dam*^h *dam*-x double mutants (previously designated *u*^R*PI*)(13), which have a Dam⁻ phenotype. Since the enzyme synthesized by these double mutants is methylation-defective, there should be no barrier to cloning the genes. By sequencing two independent, mutant *dam*^h *dam*-x genes (originating from the same *dam*^h parent), and comparing their sequences to that of the *dam*⁺ gene, we expected to be able to determine the common *dam*^h mutation and the individual *dam*⁻ mutations. This strategy proved to be successful; the *dam*^h mutation was found to change Pro¹²⁶ to Ser¹²⁶. We also report another mutation, *dam*^c, which changes Phe¹²⁷ to Val¹²⁷; the Dam^c enzyme methylates GATC in C-DNA, but not in hmC-DNA. Because these mutations occur in homology region III shared by T4 Dam, *E. coli* Dam, and M-DpnII, we propose that this region is involved in DNA sequence recognition. In addition, we report that four different amino acids can be substituted at position 126 and all produce a functional Dam^h-like methyltransferase capable of methylating C-DNA and hmC-DNA at both canonical and non-canonical sites.

The sequence of the T4 *dam*⁺ gene cloned in our laboratory (2) was compared to that determined by another laboratory (14). We observed five nucleotide differences, responsible for three amino acid differences. Despite these differences, both Dam⁺ enzymes are active on hmC-DNA and methylate to similar extents; but, more subtle changes in enzyme activity may exist.

MATERIALS AND METHODS

Bacteria, bacteriophage, and plasmids. *Bacteria:* *E. coli* K704 (F⁻ *mcrA mcrB met gal supE* R_K⁺M_K⁺); *E. coli* F⁺1100 *mcrA mcrB* R_K⁻M_K⁺ *supE endI thi* (15); *E. coli* SK1036 *dam*-4 (16); *E. coli* GM2971 (F⁻ *mrr*⁻ *hsd20* R_B⁻M_B⁻ *thi ara14 leu proA2 lacY1 galK2 rpsL20* (*str*^R) *xyl5 mtl1 supE44 endA dam13::Tn9 Cm*) was from M.G. Marinus; *E. coli* RZ1032 *dut*⁻ *ung*⁻ was from L. Lindahl (17). *Bacteriophages:* T6 *αgt*₄₁⁻; T2 *αgt*₁⁻ *dam*^h *dam*-1; T4 *αgt*₁⁻ *βgt*₂₇⁻ *dam*⁺ and T4 *αgt*₁⁻ *βgt*₂₇⁻ *dam*^h were from H. Revel (15); T4 *αgt*₁⁻ *βgt*₂₇⁻ *dam*^h *dam*-1 and T4 *αgt*₁⁻ *βgt*₂₇⁻ *dam*^h *dam*-3 were isolated in our laboratory; M13rv1 was from R. Myers (18). *Plasmids:* pGC1 (19); pACYC184 (20); pSSH-10 contains the T4 *dam*⁺ gene in pBR322 (our collection); pZAM126, pZAM126ACYC and pZM11 contain the T4 *dam*⁺ gene in pUC18, pACYC184, and pGC1 respectively (this paper); pLL1, containing the *supD* gene (21), was from L. Lindahl; pGF1B-1 containing suppressors for alanine, cysteine, phenylalanine, glutamic acid, histidine, or glycine (22,23) were from J.H. Miller. The T4 *dam*⁺ gene was cloned in this lab from T4*am*N55×5(gene 42), *am*E51(gene 56),*nd*28 (gene *denA*), D2a2(gene *denB*), *alc*10 (2).

Chemicals and Media LB medium is described in Maniatis, et al. (24). Ampicillin was added to media to 50 μg/ml, tetracycline to 15 μg/ml, and 2-aminopurine (Sigma) to 400 μg/ml. S-adenosyl-L-[methyl-³H]methionine (AdoMet; 69.9 Ci/mmol; 1 mCi/ml) and [α-³⁵S]-dATP (600 Ci/mmol) were from Amersham. PD-10 columns were from Pharmacia. TE is 10 mM Tris-HCl (pH 8.0), 1.0 mM Na₂EDTA (pH 8.0). Column buffer (CB) was 20 mM NaH₂PO₄ (pH 6.5), 0.5 mM Na₂EDTA, 10 mM 2-mercaptoethanol, 40 mM NaCl, 10%(v/v) glycerol. CBP is CB with 2 mM phenylmethyl-sulfonylfluoride (PMSF). Purification of cold AdoMet is described by Gefter, et al. (25).

General Methods. Phage DNA isolation was previously described (26). DNA sequencing was by the dideoxy method (27) with [α - 35 S]-dATP as the labeled nucleotide. Restriction digests, DNA fragment isolation, ligations, transformations, and plasmid DNA purification were carried out as described by Maniatis et al. (24).

Oligonucleotide Primers. The oligonucleotides 5'CCAAACTAAGTAGTAAAATTTCC 3' (for the mutagenic primer) and 5'CTGGCACGCGCTGGACGCG 3' (for the 2nd primer) were synthesized using the phosphoramidite method. The oligonucleotides were heated in concentrated (37%) ammonium hydroxide at 50°C for approximately four hours and chromatographed on a PD-10 column according to the manufacturer's (Pharmacia) directions.

Preparation of single-stranded (ss) DNA from pZM11. A 5 ml culture of *E. coli* RZ1032 containing plasmid pZM11 was grown to 8×10^7 cells per ml in LB broth supplemented with 0.25 μ g/ml uridine. Phage M13rv1 was added at an input ratio of 10 per cell and the culture was incubated for 7 hr at 37°C. Cells were pelleted by centrifugation at $5,900 \times g$; NaCl (0.5 M) and PEG-8000 (7% w/v) were added to the supernatant; after 15 min at 25°C, the phage were pelleted by centrifugation at $12,000 \times g$ for 10 min and resuspended in 0.4 ml TE. Deproteinization was by successive extractions with water-saturated phenol and chloroform-isoamyl alcohol (24:1). After overnight precipitation in 70% ethanol at -20°C, the ssDNA was collected by centrifugation, washed once in 70% ethanol, and dissolved in 10 mM Tris-HCl (pH 8.0), 0.1 mM Na₂EDTA (pH 8.0).

Construction of a Polypeptide Chain Terminating (Amber) Codon at Residue 126 Using Site-Directed Mutagenesis. The T4 *dam*⁺ gene was cloned into the vector pGC1, which contains an M13 origin of replication, so that ssDNA could be prepared for use as a template in site-directed mutagenesis. This construction was designated pZM11. The method of Kunkel, et al.(17) was followed except that pZM11 was used to generate a ssDNA template and two primers were annealed prior to polymerization with *E. coli* PolIk (Bethesda Research Laboratory). Competent cells were transformed with an aliquot of the polymerization reaction mixture. Transformants were screened for loss of Dam activity by their sensitivity to 2-aminopurine (10). DNA sequencing confirmed the presence of the amber mutation. The mutated gene was then subcloned into pACYC184, designated pZAM126ACYC, so as to be compatible with colE1 plasmids containing cloned suppressor genes.

Partial Purification of Dam Enzymes. *E.coli* SK1036 containing either pSSH-10 or pZAM126ACYC and plasmids carrying different suppressor genes were grown in appropriate antibiotic-containing medium at 37°C (or at 30°C for pLL1) to OD₆₀₀ = 0.5. Cells from a one liter culture were harvested by centrifugation. The cells were suspended in 25 ml TE, harvested again, suspended in 10 ml CBP, and placed at -20°C overnight. After thawing, the cells were lysed either in a French press (at 10,000 psi) or by sonication. After low speed ($27,000 \times g$ for 30 min) and high speed ($144,000 \times g$ for 2 hr) centrifugation, the supernatant was fractionated in ammonium sulfate; proteins precipitated between 40–70% were dissolved in 10 ml CBP and dialyzed overnight at 4°C against CB. The dialysate was applied to a DEAE Sephacel (Pharmacia) column (3 ml bed volume) previously equilibrated with CB. Proteins were eluted by a NaCl gradient (40–500 mM) in CB. One ml fractions were collected and selected samples assayed for DNA methyltransferase activity. The various Dam proteins exhibited different chromatographic properties; e.g. Dam⁺ (Pro¹²⁶) eluted at approximately 150 mM NaCl; Dam (Ala¹²⁶)

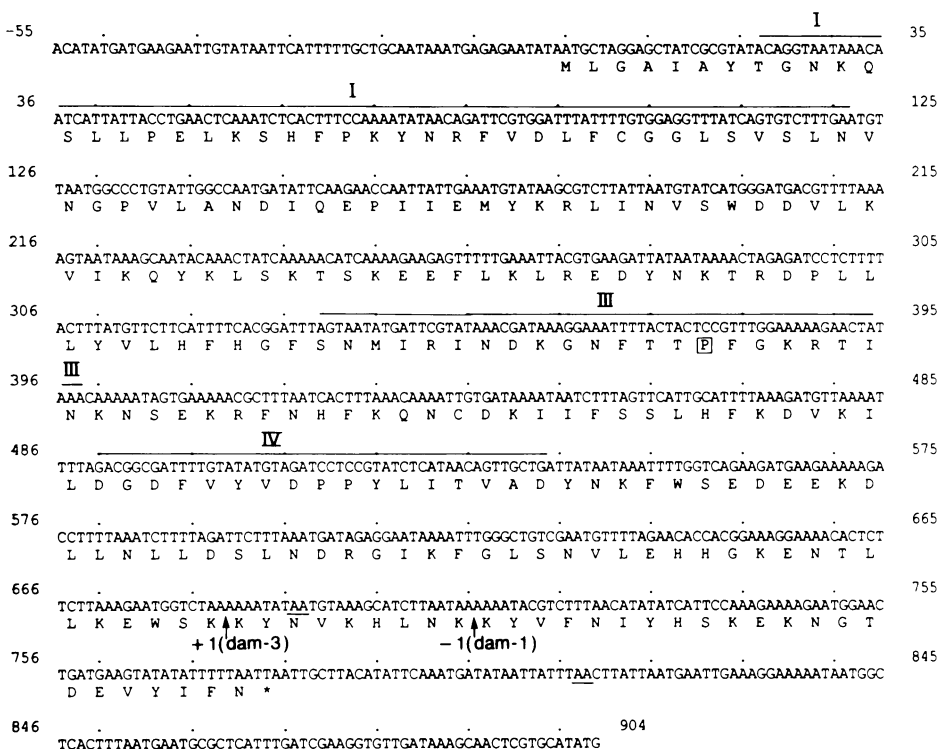


Figure 1. The nucleotide sequence of the T4 *dam*⁺ gene and the derived amino acid sequence of the Dam⁺. Residue 126 (Pro), which is serine in Dam^h, and residue 127 (Phe) which is valine in Dam^c, are enclosed in boxes. The locations of the *dam*-1 and *dam*-3 frameshift mutations are indicated by arrows. The termination codons that result from the *dam*⁻ frameshifts are underlined. Regions of amino acid sequence similarity (I, III, and IV) among isoschizomeric m⁶A-MTases are overlined.

eluted at approximately 80 mM NaCl; and Dam (Gly¹²⁶), Dam (Ser¹²⁶), and Dam (Cys¹²⁶) did not bind to the column matrix.

In Vitro DNA Methyltransferase Assays. DNA methyltransferase activity was assayed at 30°C as follows: the reaction mixtures (0.2 ml total volume) contained 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 5 mM Na₂EDTA (pH 8.0), 5 mM 2-mercaptoethanol, 20 µg/ml DNA, [methyl ³H]-AdoMet (0.2 µCi/2.8 pmol plus 5 µM cold AdoMet), and 1–5 µl of partially purified protein. Optimal assay conditions for pH, NaCl concentration, and AdoMet (K_m = 1.4 µM) were established using the Dam⁺ enzyme.

RESULTS

Isolation of T4 *dam*^h *dam*-x mutants. The increased level of methylation due to the *dam*^h mutation protects non-glucosylated T4 *αgt*⁻ *βgt*⁻ *dam*^h phage against P1-restriction (6). Therefore, the titer of *dam*^h phage assayed on the restrictive, P1-lysogenic host, K704(P1), is approximately equal to the titer assayed on the permissive, non-lysogenic parent, K704. To obtain spontaneous second-site mutants, *dam*^h *dam*-x (which have a Dam⁻ phenotype),

T4 *dam*^h plaques on K704 were individually picked and spotted onto lawns of K704 and K704(P1) to screen for the loss of infectivity on K704(P1). We observed a high frequency of the double mutant (10^{-3}), suggesting that unmethylated mutants may have a growth advantage over the hypermethylating, parental, *dam*^h phage. This was in agreement with the observed frequencies of T2 *dam*^h *dam*-x mutants (13). Virion DNA isolated from two independent mutants was sensitive to cleavage by *Mbo*I, but not to cleavage by *Dpn*I (data not shown), which is diagnostic of DNA lacking m⁶A in the sequence, GATC (or GAThC). The mutants were designated *dam*^h *dam*-1 and *dam*^h *dam*-3.

Cloning and sequencing dam^h dam-x mutant genes. Previous analyses (2,14) showed that the T4 *dam* gene is located on a 2.1 kb *Xba*I fragment. The fragment was isolated from *Xba*I genomic digests of both T4 α gt⁻ β gt⁻ *dam*^h *dam*-1 and T4 α gt⁻ β gt⁻ *dam*^h *dam*-3, and ligated into the *Xba*I site of the vector pUC18. *E. coli* F⁺1100 (a host that does not restrict non-glucosylated hmC-DNA) transformants containing the cloned fragment were identified; the recombinant plasmids were designated pZM41 and pZM43, respectively.

We anticipated that the *dam*^h mutation could be identified by comparing the nucleotide sequences of two independent *dam*^h *dam*-x mutants against the wild-type *dam*⁺ sequence. According to this strategy, the double mutants should share a common mutation (*dam*^h), as well as additional, but different, alterations (*dam*-1 and *dam*-3). To our surprise, the two *dam*^h *dam*-x sequences contained six nucleotide substitutions compared to the published *dam*⁺ sequence (14), as well as two different frameshifts (+1 and -1). This unexpected finding prompted us to sequence our parental *dam*⁺ gene (2) which was cloned from a different T4 DNA source from that used by others (14). The sequence of our *dam*⁺ gene differed from the two *dam*^h *dam*-x sequences by only a single common base substitution and a unique frameshift (+1 or -1) mutation (Fig. 1). The common substitution identified as the *dam*^h mutation, was a C to T transition resulting in a Pro¹²⁶ to Ser¹²⁶ change. Additional support for this conclusion came from the analysis of a T2 *dam*^h*dam*-x mutant; it also possessed a Pro¹²⁶ to Ser¹²⁶ coding change (not shown).

Effect of different amino acids at residue 126. To study the effect of other amino acids at residue 126, a polypeptide chain terminating amber (UAG) mutation was introduced at this codon by site-directed mutagenesis (see MATERIALS AND METHODS). Various amino acids were then inserted in vivo at this position by the action of different cloned tRNA suppressors. Insertion of either phenylalanine, histidine, or glutamic acid resulted in loss of detectable enzyme activity (not shown). However, insertion of serine (the *dam*^h change), alanine, cysteine, or glycine all gave active enzyme. The latter four enzymes were partially purified and compared to wild-type Dam⁺ enzyme with respect to their ability to methylate various DNA substrates. The yield of enzyme activity differed in each case, perhaps because of differences in the efficiencies of suppression, enzyme stabilities, and intrinsic activities. However, by using a constant amount of protein for a given enzyme, we could compare the relative ability of that enzyme to methylate different substrates (Fig. 2).

With DNA containing unmethylated GATC or GAThC, the canonical sites, the Dam⁺ (Pro¹²⁶) methyltransferase preparation exhibited a relatively higher activity than Dam^h (Ser¹²⁶) or Dam (Gly¹²⁶). When the substrate was *dam*⁺ hmC-DNA, containing methylated (Gm⁶AThC) sites, the Ser¹²⁶ and Gly¹²⁶ variant enzymes exhibited about twenty-fold higher rates of methylation than did Dam⁺ (Table 1). The Cys¹²⁶ and Ala¹²⁶ enzymes behaved in a similar manner to Dam^h (not shown). These results indicate that Dam⁺ methylates primarily GATC and GAThC, which is in agreement with the finding

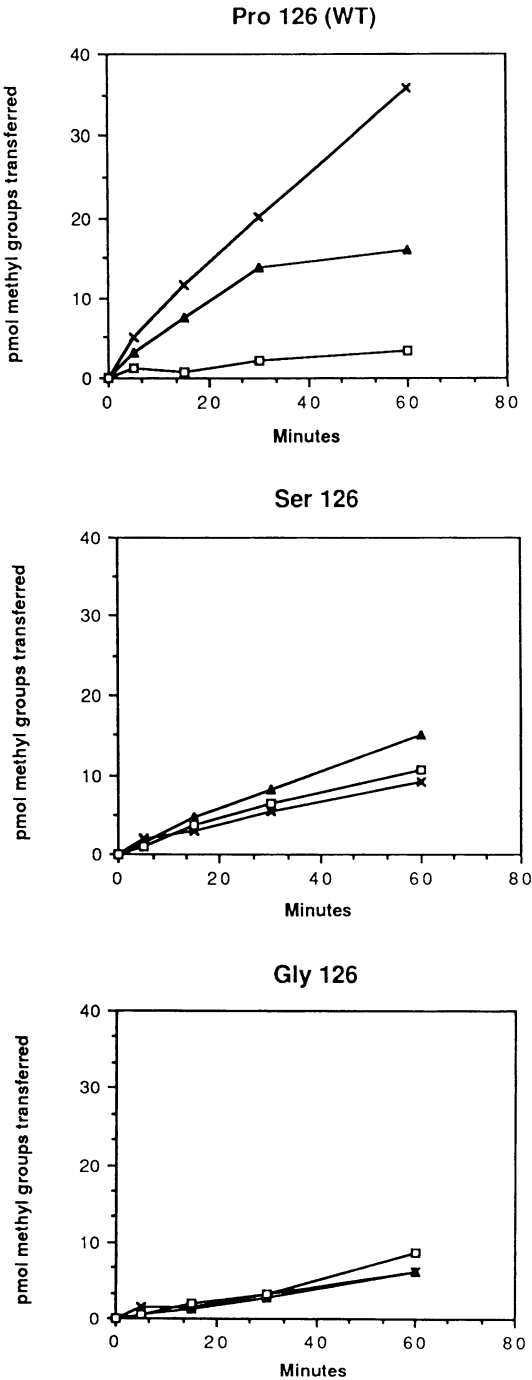


Table I. Comparison of relative methylation rates by Dam enzymes having different amino acids at residue 126^a

Amino Acid 126	Relative Methylation Rate on:	
	<i>dam</i> ⁺ DNA	<i>dam</i> ⁻ DNA
proline (<i>Dam</i> ⁺)	0.05	0.7
serine (<i>Dam</i> ^h)	1.1	1.5
alanine	2.5	1.5
cysteine	0.3	1.1
glycine	0.8	0.8

^a Relative methylation rates were calculated from the slopes (15–30 minutes) in Figure 2; the slopes with *dam*⁺-hmc DNA and *dam*⁻-hmc DNA were divided by the slope with Calf Thymus DNA.

of Doolittle and Sirotkin (27) and with our own findings (29, 30, S.L. Schlagman and S. Hattman, in preparation). In contrast, the Dam derivatives containing serine, glycine, alanine, or cysteine at residue 126 readily methylate non-canonical sites; moreover, they retain the ability to methylate GATC and GAThmc, as shown by *Mbo*I and *Dpn*II cleavage analysis (not shown). These findings support further the notion that the Pro¹²⁶ to Ser¹²⁶ change is the result of the *dam*^h mutation, and they demonstrate that other substitutions at position 126 can also alter Dam sequence-specificity.

A Phe to Val change at residue 127 alters sequence specificity. In the course of other experiments with the cloned *dam*⁺ gene, a spontaneous mutation arose that allows methylation of GATC in C-DNA but not in hmc-DNA (not shown). Sequence analysis revealed that the mutant, designated *dam*^c, has a single nucleotide difference that changes amino acid 127 from Phe to Val. Thus, another single amino acid substitution in homology region III changes the substrate recognition of the T4 Dam m⁶A-MTase.

DISCUSSION

T4 *dam*^h (and T2 *dam*^h) mutants hypermethylate their DNA compared to the parental *dam*⁺ forms. The results of our cloning and sequencing studies indicate that the *dam*^h mutation causes a Pro¹²⁶ to Ser¹²⁶ alteration in the Dam protein. In addition, we demonstrate that other amino acids at residue 126 can also alter the sequence specificity of the Dam protein. Five different amino acids (proline, serine, alanine, cysteine, and glycine) at residue 126 produce an active m⁶A-MTase, while replacement with phenylalanine, glutamic acid or histidine at that position appears to render the enzyme inactive (the stability of the latter three derivatives is unknown at this time). The five amino acids that result in a *Dam*^h-like enzyme tend to be found in turns, and, Chou-Fasman secondary structure predictions place residues 126 and 127 in a turn (31). This suggests

Figure 2. Methylation of substrate DNA's by partially purified T4 m⁶A-MTases. At intervals, samples were withdrawn from scaled-up reaction mixtures (see MATERIALS AND METHODS) and precipitated in cold 5% trichloroacetic acid, collected on GF/C glass filter disks and washed with 5% trichloroacetic acid. After drying, the radioactivity was counted in an LKB 1209 Rackbeta scintillation counter. The specific activity of the [³H]-AdoMet was 182 cpm/pmol. The values on the ordinate were corrected by subtracting values (1.5 to 2.0 pmol, depending on the enzyme) of reactions lacking DNA; these values were the same at 5 and 60 min. Calf thymus DNA, (x—x); *dam*⁻ hmc DNA (▲—▲); *dam*⁺ hmc DNA, (□—□).

that contact between the Dam protein and its DNA target sequence may involve some other nearby amino acid residue(s). On the other hand, if residue 126 is directly involved in protein-DNA interaction, this would be either through hydrogen bonding (in the case of serine) or hydrophobic interaction (in the case of cysteine, glycine, proline, or alanine).

The *dam^c* mutation, which substitutes Val¹²⁷ for Phe¹²⁷, results in an enzyme that is able to methylate GATC in C-DNA, but not in hmC-DNA. The fact that adjacent amino acid substitutions in homology region III (12), a conserved region found in four isoschizomeric m⁶A-MTases (*E. coli* Dam, T2 Dam, T4 Dam and M-DpnII) capable of methylating GATC, result in enzymes with altered DNA recognition ability implicates this region in DNA sequence discrimination. It is also interesting to note that the Pro¹²⁶-(Phe/Tyr)¹²⁷ motif is highly conserved among a group of m⁶A-MTases that have different methylation target sequences (31).

The T2 Dam⁺ protein is able to methylate C-DNA and hmC-DNA to higher levels than T4 Dam⁺ is (6, 26). Sequence comparison of the two enzymes indicate that there are three amino acid differences (26); two are located in homology region I and the third (a conservative change) is located downstream of homology region IV. We are presently trying to determine which of the differences is responsible for the altered sequence specificity of T2 Dam⁺. It appears likely that homology region I may be involved in sequence recognition. If this notion is correct, then regions I and III may be in close proximity to one another in the native protein. Region IV may also be in the active site because this domain contains the (Asp/Asn)-Pro-Pro-(Tyr/Phe) motif, common to almost all m⁶A-MTases, and thought to be involved in adenine recognition or AdoMet binding (32).

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*To whom correspondence should be addressed

⁺Present address: The Biological Laboratories, Harvard University. 16 Divinity Avenue, Cambridge, MA 02138, USA

REFERENCES

1. Hattman, S. Van Ormondt, H. and deWaard, A. (1978) *J. Mol. Biol.*, **119**, 361–376.
2. Schlagman, S. L. and Hattman, S. (1983) *Gene*, **22**, 139–156.
3. Van Ormondt, H., Gorter, J., Havelaar, K. J. and deWaard, A. (1975) *Nucleic Acids Res.*, **2**, 1391–1400.
4. Hattman, S. (1970) In P. D. Boyer (ed.) *The Enzymes*, Academic Press, Vol. 14, p. 514–518.
5. Revel, H. R. and Luria, S. (1970) *Ann. Rev. Genet.*, **4**, 177–192.
6. Hattman, S. (1970) *Virology*, **42**, 359–370.
7. Brooks, J. E. and Hattman, S. (1978) *J. Mol. Biol.*, **126**, 381–394.
8. Hattman, S., Brooks, J. E. and Masurekar, M. (1978) *J. Mol. Biol.*, **126**, 367–380.
9. Bächli, C. B., Reiser, J. and Pirrotta, V. (1979) *J. Mol. Biol.*, **128**, 143–163.
10. Marinus, M. B. and Morris, N. R. (1973) *J. Bacteriol.*, **114**, 1143–1150.
11. Geier, G. E. and Modrich, P. (1979) *J. Biol. Chem.*, **254**, 1995–2006.
12. Hattman, S., Wilkinson, J., Swinton, D., Schlagman, S., Macdonald, P. M. and Mosig, G. (1985) *J. Bacteriol.*, **164**, 932–937.
13. Revel, H. R. and Hattman, S. (1971) *Virology*, **45**, 484–495.
14. Macdonald, P. M. and Mosig, G. (1984) *EMBO J.*, **3**, 2863–2871.

15. Revel, H. R. (1967) *Virology*, **32**, 688–701.
16. Herman, G. E. and Modrich, P. (1981) *J. Bacteriol.*, **169**, 644–646.
17. Kunkel, T. A., Roberts, J. D. and Zakour, R. A. (1987) *Methods in Enzymol.*, **154**, 367–382.
18. Levinson, A., Silver, D. and Seed, B. (1984) *J. Molec. Appl. Genet.*, **2**, 507–517.
19. Myers, R. M., Lerman, L. S. and Maniatis, T. (1985) *Science*, **229**, 242–247.
20. Chang, A. C. Y. and Cohen, S. N. (1978) *J. Bacteriol.*, **134**, 1141–1156.
21. Zengel, J. M. and Lindahl, L. (1981) *J. Bacteriol.*, **145**, 459–465.
22. Normanly, J., Masson, J.-M., Kleina, L. G., Abelson, J. and Miller, J. H. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 6548–6552.
23. Cupples, C. G. and Miller, J. H. (1988) *Genetics*, **120**, 637–644.
24. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
25. Gefter, M., Hausmann, R., Gold, M. and Hurwitz, J. (1966) *J. Biol. Chem.*, **241**, 1995–2006.
26. Miner, Z. and Hattman, S. (1988) *J. Bacteriol.*, **170**, 5177–5184.
27. Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.
28. Doolittle, M. M. and Sirotkin, K. (1988) *Biochim. Biophys. Acta*, **949**, 240–246.
29. Schlagman, S. L., Hattman, S. and Marinus, M. G. (1986) *J. Bacteriol.*, **165**, 896–900.
30. Schlagman, S. L., Miner, Z., Fehér, Z., and Hattman, S. (1988) *Gene*, **73**, 515–528.
31. Guschlbauer, W. (1988). *Gene*, **74**, 211–214.
32. Chandrasegaran, S. and Smith, H. O. (1987) In Sarma, R. H. and Sarma, M. H. (eds.) *Structure and Expression: from Proteins to Ribosomes*, Adenine Press, Schenectady, NY, Vol. I, 149–156.

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